

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1-30. (Canceled)

31. (Currently Amended) A method for detecting the presence of ~~anti-HLA~~ **anti-MHC** antibodies in a sample, the method comprising the steps of:

providing a substrate ~~having a:~~

providing a pool of functionally active, **recombinantly produced,**
truncated individual soluble ~~HLA molecule linked thereto~~ **MHC**
trimolecular complexes, each trimolecular complex
comprising a recombinant, soluble MHC heavy chain allele,
beta-2-microglobulin, and endogenously loaded peptide, the
functionally active, **recombinantly produced, truncated**
individual soluble ~~HLA molecule~~ **MHC trimolecular complexes**
being purified substantially away from other proteins such that the
individual soluble ~~HLA molecule maintains~~ **MHC trimolecular**
complexes maintain the physical, functional and antigenic
integrity of the native ~~HLA molecule, the functionally active,~~
~~individual soluble HLA molecule being directly or indirectly linked to~~
~~the substrate~~ **MHC trimolecular complex, wherein the**

individual soluble MHC trimolecular complexes are produced
by a method comprising the steps of:
isolating mRNA from a source, wherein the mRNA encodes
at least one MHC heavy chain allele;
reverse transcribing the mRNA to obtain cDNA;
identifying an individual MHC heavy chain allele in the cDNA;
PCR amplifying the individual MHC heavy chain allele in a
locus-specific manner to produce a PCR product having
the coding regions encoding cytoplasmic and
transmembrane domains of the individual MHC heavy
chain allele removed such that the PCR product
encodes a truncated, soluble form of the individual
MHC heavy chain molecule;
cloning the PCR product into a mammalian expression
vector, thereby forming a construct that encodes the
individual soluble MHC heavy chain molecule;
transfecting a mammalian cell line with the construct to
provide a mammalian cell line expressing a construct
that encodes a recombinant, individual soluble MHC
heavy chain molecule, wherein the mammalian cell line
is able to naturally process proteins into peptide

ligands for loading into antigen binding grooves of MHC molecules;

culturing the mammalian cell line under conditions which allow for expression of the recombinant individual soluble MHC heavy chain molecule from the construct, such conditions also allowing for endogenous loading of a peptide ligand into the antigen binding groove of each individual soluble MHC heavy chain molecule in the presence of beta-2-microglobulin to form the individual soluble MHC trimolecular complexes prior to secretion of the individual soluble MHC trimolecular complexes from the cell; and

purifying the individual, soluble MHC trimolecular complexes substantially away from other proteins, wherein the individual soluble MHC trimolecular complexes maintain the physical, functional and antigenic integrity of the native MHC trimolecular complex;

linking at least one soluble MHC trimolecular complex to a substrate, wherein the at least one soluble MHC trimolecular complex is directly or indirectly linked to the substrate, and wherein the at least one soluble MHC

trimolecular complex linked to the substrate retains the physical, functional and antigenic integrity of the native MHC trimolecular complex;

providing a sample;

reacting the sample with the substrate having the ~~functionally active, individual soluble HLA molecule~~ **at least one MHC trimolecular complex** linked thereto;

washing the substrate to remove unbound portions of the sample;

reacting the substrate having the ~~functionally active, individual soluble HLA molecule~~ **at least one MHC trimolecular complex** linked thereto with means for detecting anti-HLA **anti-MHC** antibodies; and

determining that anti-HLA **anti-MHC** antibodies specific for the HLA molecule **individual MHC molecule** are present in the sample if the means for detecting anti-HLA **anti-MHC** antibodies is positive.

32. (Currently Amended) The method of claim 31 wherein, in the step of providing a substrate ~~having a functionally active, individual soluble HLA molecule linked thereto~~, the substrate is a solid support.

33. (Currently Amended) The method of claim 32 wherein the solid support is selected from the group consisting of a well, a bead, a membrane, an ELISA plate, **and** a matrix, ~~and combinations thereof~~.

34. (Currently Amended) The method of claim 33 wherein the bead is selected from the group consisting of a flow cytometry bead, a ~~Luminex bead, a Dynabead,~~ a magnetic bead and combinations thereof, and wherein the membrane is selected from the group consisting of a nitrocellulose membrane, a PVDF membrane, a nylon membrane, and acetate derivative, and combinations thereof.

35. (Currently Amended) The method of claim 31 wherein, in the step of ~~providing a substrate having a functionally active, individual soluble HLA molecule linked thereto,~~ the functionally active, individual soluble HLA molecule **linking at least one soluble MHC trimolecular complex to a substrate, the at least one soluble MHC trimolecular complex** is indirectly attached to the substrate via an anchoring moiety.

36. (Currently Amended) The method of claim 35 wherein the anchoring moiety comprises an antibody to the functionally active, individual soluble HLA molecule **MHC trimolecular complex**.

37. (Currently Amended) The method of claim 36 wherein the antibody is selected from the group consisting of W6/32, anti-beta 2m,

~~Pan-Class I~~ **pan-Class I** or allele-specific antibodies and combinations thereof.

38. (Withdrawn – Currently Amended) The method of claim 35 wherein the anchoring moiety comprises a tail or tag attached to the functionally active, individual soluble ~~HLA molecule~~ **MHC trimolecular complex**, and the substrate is further defined as comprising an affinity reagent to which the tail or tag binds.

39. (Withdrawn) The method of claim 38 wherein the tail or tag is a histidine tag, and the affinity reagent is selected from the group consisting of nickel, copper and combinations thereof.

40. (Withdrawn) The method of claim 38 wherein the tail or tag is a biotinylation signal peptide, and the affinity reagent is avidin or streptavidin.

41. (Withdrawn) The method of claim 38 wherein the tail or tag is a VLDLr or FLAG tail, and the affinity reagent is an antibody that recognizes the VLDLr or FLAG tail.

42. (Currently Amended) The method of claim 31 wherein, in the step of providing a ~~substrate having a functionally active, individual soluble HLA molecule linked thereto, the functionally active, individual soluble HLA molecule is a Class I HLA molecule or a Class II HLA molecule~~ **pool of functionally active, recombinantly produced, truncated individual soluble MHC trimolecular complexes, the pool of functionally active, recombinantly produced, truncated individual soluble MHC trimolecular complexes are Class I or Class II MHC trimolecular complexes.**

43-44. (Canceled)

45. (Currently Amended) The method of claim **[44] 31** wherein, in the step of isolating ~~HLA-allele~~ mRNA from a source, the source is selected from the group consisting of mammalian DNA and an immortalized cell line.

46. (Currently Amended) The method of claim **[44] 31** wherein, in the step of ~~inserting the truncated~~ **cloning the** PCR product into a mammalian expression vector, the mammalian expression vector contains a promoter that facilitates increased expression of the truncated PCR product.

47. (Currently Amended) The method of claim [44] **31** wherein, in the step of ~~electroporating the plasmid containing the truncated PCR product into at least one suitable host cell, the suitable host cell~~ **transfecting a mammalian cell line, the mammalian cell line** lacks expression of Class I HLA **MHC** molecules.

48. (Currently Amended) The method of claim [44] **31** wherein, in the step of ~~amplifying the allelic cDNA by PCR, the class-I specific primer~~ **PCR amplifying the individual MHC heavy chain allele, a primer utilized in the PCR amplification** includes a sequence encoding a tail such that the soluble HLA **MHC heavy chain** molecule encoded by the truncated PCR product contains a tail attached thereto that facilitates in purification of the soluble HLA ~~molecules~~ **MHC trimolecular complexes** produced there from or facilitates in direct binding of the soluble HLA ~~molecules~~ **MHC trimolecular complexes** to the substrate.

49. (Currently Amended) The method of claim [44] **31** wherein, in the step of ~~amplifying the allelic cDNA by PCR, the at least one class-I specific primer~~ **PCR amplifying the individual MHC heavy chain allele, a 3' primer utilized in the PCR amplification** includes a stop codon incorporated into ~~a 3' primer~~ **therein**.

50. (Currently Amended) The method of claim ~~[44]~~ **31** wherein, in the step of purifying the individual, soluble ~~HLA~~ molecules **MHC trimolecular complexes** substantially away from other proteins, the functionally active, individual soluble ~~HLA~~ molecule is **MHC trimolecular complexes** purified by affinity chromatography and fractionation.

51. (Currently Amended) The method of claim 50 wherein the affinity chromatography utilizes a reagent selected from the group consisting of W6/32 antibodies, anti- β 2m antibodies, ~~Pan-Class-I~~ **pan-Class I** antibodies or allele-specific antibodies, and combinations thereof.

52-59. (Canceled)

60. (Previously Presented) The method of claim 31 wherein, in the step of providing a sample, the sample is selected from the group consisting of serum, tissue, blood, cerebrospinal fluid, tears, saliva, lymph, dialysis fluid, organ or tissue culture derived fluids, fluids extracted from physiological tissues, and combinations thereof.

61. (Currently Amended) The method of claim 31 wherein, in the step of reacting the substrate having the ~~functionally active, individual soluble HLA molecule~~ **at least one MHC trimolecular complex** linked thereto with means for detecting ~~anti-HLA~~ **anti-MHC** antibodies, the means for detecting ~~anti-HLA~~ **anti-MHC** antibodies is a labeled anti-human antibody recognizing human IgG, IgM or IgA antibodies.

62-92. (Canceled)